To: Peters, Emily (MPCA)[Emily.Peters@state.mn.us]

From: Erickson, Russell

Sent: Wed 2/19/2014 4:44:12 PM

Subject: RE: anova results

Emily:

Some of the issues here are where my advice would not be definitive and where you should try to get feedback from a statistician. However, with that caveat, here are some comments:

- (1) Regarding the ANCOVA, only sulfide should be treated as a covariate "Test" should still be treated as a categorical variable in a mixed model.
- (2) Regarding the ANCOVA, treating sulfide as a covariate means that similar levels across tests are not considered the same treatment, which precludes pair-wise comparisons (and it is not possible to have the same treatment both as a category and a covariate in the same analysis). However, The ANCOVA is still establishing a significant sulfide effect, and there is a monotonic, nearly linear data trend consistent with the applied model. Therefore, this pairwise comparison for the highest treatment in the analysis would really not be necessary there is a sulfide effect and at treatment 2 it is about 30%. Some post hoc test treating Treatment 2 as the same treatment and comparing it to the controls might be possible, but you would need that consultation with a statistician regarding this (and you already did that in the pooled ANOVA).
- (3) Regarding the dependent variable, one additional change that might be useful for communicating the results is to use % weight gain ((FinalWt-InitialWt)/InitialWt*100). This doesn't change the stats, but directly addresses the magnitude of the effect at each treatment in a way that might be more meaningful to your audiences. Sorry I didn't mention it as a possibility earlier. However, this change is simply cosmetic it would not modify the significance levels for the treatments (it would modify them for the test effect) and this % effect is easily calculated after the fact from your current analyses.
- (4) I would test the homogeneity of variance in these various tests, and modify the analyses accordingly. I believe that the pooled analysis without T3 and T4 would not have a problem with this, but the individual test ANOVAs might have. For example, it is possible that the marginally significant effect at Treatment 2 for the rangefinder is actually not quite this significant because the overall residual variance is reduced by the T4 data. If there are

nonhomogenous errors, a log-transform might help, but then you would have to run the tests just on weights (to avoid negative numbers), and calculate percent effect after the fact. An alternative would be to run ANOVAs on the individual tests with just the lower three treatments to test the effect at T2, but that would also reduce the test power due to lower n.

- (5) Regarding the individual vs pooled ANOVAs, the pooled ANOVA is the more germane one regarding effects at T2. After all, there are three independent tests here and effects should be judged based on the totality of the data not the significance of any one test. Running any number of individual tests with small n and limited power can continually miss a true effect which can only be detected by pooling the data. A statistician might suggest other strategies for doing this than these pooled ANOVA/ANCOVAs.
- (6) Just as you did with the regression analysis, I would run the ANCOVA test both with initial and mean sulfide. This would not affect the categorical ANOVAs (except for what sulfide is associated with an effect level), but might affect the ANCOVA significance level. This is probably unimportant, since the average for both T1 and T2 are both about half of the initial concentration. However, the application of your results will involve considering what exposure metric to use and having results for both metrics might be useful.
- (7) I would consider dropping the Tukey comparisons, at least in any final presentation. The important comparison here is treatments versus the control, so that just presenting the Dunnett's results makes things simpler and to the point. (Some specific a priori contrast are another possibility.)
- (8) For the pooled analysis, I would retain both the ANOVA and ANCOVA as part of the total analysis package. The logic of the progression of the analysis goes like this in my mind:
- (a) Individual ANOVAs on each test establish a treatment effect that is dominated by the large effects in T3 and T4 (which are obvious), but do raise the possibility of some effect at T2.
- (b) A pooled ANOVA excluding T3 and T4 as irrelevant to the issue of effects at T2 but still treating T2 as categorical (because the average initial concentrations are very close to each other) still shows a treatment effect, with pairwise comparisons showing a significant effect at T2 relative to the control.
- (c) Recognizing that sulfide is not exactly the same in T2 and does vary moderately in T1, the ANCOVA establishes that there still is a treatment effect even treating it as a covariate.

But again, if you can, please get input from a statistician on this perspective and on the other issues above.

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From: Peters, Emily (MPCA) [mailto:Emily.Peters@state.mn.us]

Sent: Tuesday, February 18, 2014 1:20 PM

To: Erickson, Russell Subject: anova results

Russ,

I'm attaching a set of ANOVAs and an ANCOVA for the hydroponic data. My interpretation is that Treatment2 is marginally significantly different from the Control ONLY in the Range Finder Test. It is not significantly different from the Control in the D1 and D2 tests. When all three tests are pooled (for C, T1, T2), Treatment 2 is significantly different from the Control. Which of these analyses do you think is most appropriate/robust?

I'm not quite sure how to interpret the ANCOVA results. Mean sulfide is a significant model term, but how would we use this to determine if Treatment 2 is different from the Control?

Should I add '	'treatment'	as a factor	in the model?	That seems	redundant???
-Emily					

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